

Effect of Dehydration on the Photosynthetic Apparatus of Sun and Shade Leaves of Laurel Forest Trees

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Z. Naturforsch. **54c**, 704–710 (1999); received December 14, 1998

Relative Water Content, Chlorophyll Fluorescence, Laurel Forest Trees, Dehydration, Sun and Shade Leaves

In this work we study the effect of reduction in relative water content (RWC) on the chlorophyll fluorescence induction kinetics as well as on the membrane integrity, measured as electrolyte leakage, in sun and shade leaves of three Canarian laurel forest trees. No differences were found among the species and type of leaves, when the slow fluorescence kinetic parameters and electrolyte leakage were analyzed, values deviated from the normal ones at 70% and 40% RWC respectively. On the contrary, the photochemical efficiency of PSII was affected at higher values of RWC in sun leaves (90% and 52% RWC depending on the species) than in shade ones (40% RWC). These results indicate that the susceptibility of PSII to water deficit is different depending on species and environmental conditions where the leaves develop.

Introduction

The effect of drought on the photosynthetic apparatus is object of current discussion. Some studies suggest high susceptibility of PSII to foliar dehydration (Govindjee *et al.*, 1981; Havaux *et al.*, 1986). However, there is some evidence that favor the view that PSII or even the whole photosynthetic apparatus is quite robust to water deficit (Kaiser, 1982, 1987; Genty *et al.*, 1987; Stuhlfauth *et al.*, 1988; Cornic and Britains, 1991; Havaux, 1992; He *et al.*, 1995; Joshi, 1995). Interpretation of the results of different experiments is complicated by the contrasting experimental conditions adopted and by the interaction between the different environmental factors influencing photochemical processes (Havaux, 1992; Newton and McBeath, 1996), besides when the leaves are exposed to high irradiance a photoinhibitory effect can be added and the rate of active oxygen formation increases or impairs the activity of antioxidative defence reactions (Smirnoff, 1993).

The aim of this study is to determine the effect of dehydration itself on the photosynthetic apparatus of sun and shade leaves of three laurel forest trees using chlorophyll fluorescence parameters and indexes as well as electrolyte leakage determinations. The experiments were done under low

light conditions to avoid interferences with photoinhibitory mechanisms.

The Macaronesian laurel forest is a relict of a humid vegetation of the Tertiary Mediterranean flora now confined to wet areas of the higher islands of this floristic region, having the typical appearance of a cloud forest (Hollermann, 1981). Since the humidity requirement may be one of the causes of forest regression, the study of the effects of leaf dehydration on the photosynthetic functions in the tree species which predominate in this formation is of considerable interest. In previous studies we found a great plasticity in the leaves of this forest (Morales *et al.*, 1996a). Leaves developed at the top of the canopy (sun leaves) are thicker and with smaller area than the ones developed in the lower part (shade leaves).

Materials and Methods

Plant material and site of investigation

Sun and shade leaves in the first year of life and completely developed of *Persea indica* (L.) Spreng, *Laurus azorica* (Seub.) Franco and *Myrica faya* Ait., were collected in spring from the laurel forest experimental plot situated at an altitude of 820–830 m a. s. l. in the Agua García Mountain, Tenerife. The climate is humid Mediterranean with

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an average annual temperature (in the last 30 years) of 14 °C, relative humidity of 80% and precipitation of 720 mm (more details in Morales *et al.*, 1996b, c and Jiménez *et al.*, 1999). A 20 m high scaffolding tower gave us access to the leaves which were taken from the upper and lower canopy respectively. They were immediately transferred to the laboratory where they were thoroughly washed in distilled water, and hydrated to water saturation overnight.

Dehydration experiments

The experiments consisted in determining the light induced fluorescence transients in the same leaves at different levels of relative water content (RWC). For each experiment, 5 detached leaves of each species and condition (sun and shade) were maintained at room conditions (20–22 °C, and 70–80% RH and very low light, 5–10 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$) for a specific period to obtain each level of RWC. Besides, 5 leaves were hydrated to water saturation as control. Additional leaves were kept at the same room conditions and were used for electrolyte leakage measurements (5 for each measurement).

Rehydration experiments

Additionally leaves were initially dehydrated as previously described and rehydrated (overnight) when they attained appropriate values of RWC. Afterwards it was proceeded as with the former leaves.

Determination of dehydration

RWC was expressed as 100 times the ratio of the water content at each time and the water content at saturation. Leaf water content was determined from the same leaves used for fluorescence kinetic studies, by estimating the fresh weight immediately after each transient and dry weight at the end of the experiment.

Fluorescence measurements

For determination of light induced fluorescence transients, the leaves were covered for 30 min with a small leafclip to be dark adapted and the complete transient was monitored at room temperature with a portable fluorometer (Plant Effi-

ciency Analyser, PEA, Hansatech, U.K). The record length was of 120 s and the light level of 2400 $\mu\text{mol m}^{-2} \text{ s}^{-1}$.

Electrolyte leakage measurements

5 leaves at the same RWC of that used for fluorescence determinations were taken for each electrolyte leakage measurement. 0.1 g of fresh weight from the middle section of each leaf was put in 3 ml distilled water for 24 h and then the electrolyte leakage was measured (C1) with a portable Conductivity meter (Dist 4, Hanna Instruments, Mauritius). Later, the same sections were plunged into liquid nitrogen, and stored for 24 h in a freezer, and then conductivity was measured again in the same way (C2). This was assumed to remove all residual electrolytes from the leaf tissues (Raymond *et al.*, 1986). C1 and C2 in control water saturated leaf sections (C'1 and C'2, respectively) were also measured. The results were expressed with the index of injury ($I_t\%$):

$$I_t = (R_m - R_c)/(1 - R_c),$$

where $R_m = C1/(C1 + C2)$ and $R_c = C'1/(C'1 + C'2)$. I_t will be near "0" in undamaged tissues and near "100" in totally damaged ones (Flint *et al.*, 1967)

Results

No significant differences in the dehydration rates of sun and shade leaves of the three studied species could be seen under experimental conditions (Fig. 1), with the unique exception of *L. azorica* sun leaves which dehydrated more slowly than shade ones when they attained values of RWC below 60%.

The ratio of variable to maximal fluorescence (F_v/F_m), as a measurement of potential photochemical efficiency of PSII, decreased with decrease of leaf RWC in the three studied species but the decrease was greater in sun leaves mainly of *Persea indica* and *Laurus azorica*. The photosynthetic apparatus of these leaves was affected when the RWC attained 90% while in shade leaves it was unaffected until about 40% RWC. In *Myrica faya* the differences between sun and shade leaves were not so pronounced since sun leaves remained unaffected until 52% RWC (Fig. 2).

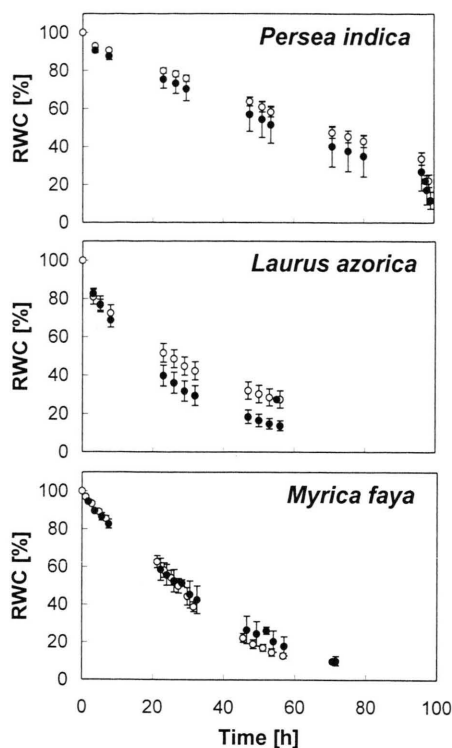


Fig. 1. Decrease of the relative water content (RWC) with the time in detached sun (open symbols) and shade (closed symbols) leaves of the three studied species. Each point represents the mean values of RWC on the same five different leaves maintained at room conditions (20–22 °C, and 70–80% RH) and very low light (5–10 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$).

We studied the slow kinetic parameters, as the vitality index (Lichtenthaler, 1988) R_{fd} , ($F_m - F_s / F_s$, F_s being the steady state fluorescence) and the fluorescence decrease ratio F_{dr} ($F_m - F_s / F_m$) showing that all leaves (In Fig. 3 we show *Persea indica* sun and shade leaves) started to be affected at RWC of about 70%, although in sun leaves the decrease was more pronounced.

While in *Myrica faya* and shade leaves of the other two species (Fig. 4), the values of minimal fluorescence (F_o) remained unaffected until very low values of RWC, sun leaves of *P. indica* and *L. azorica* showed an increase at similar values as we observed before with F_v/F_m ratio.

The effect of the rehydration in detached sun and shade leaves of *Persea indica* can be seen in Fig. 5. The asterisks show the effect of the RWC decrease as shown in Fig. 2. When sun leaves started to deviate from the normal values of F_v/F_m

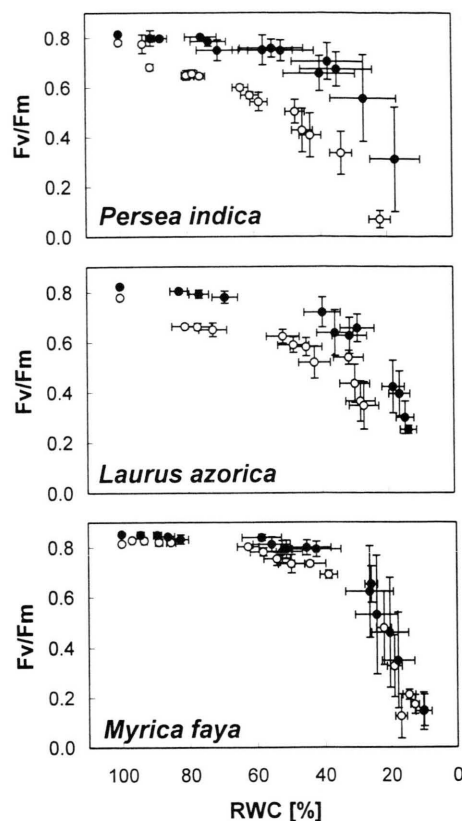


Fig. 2. Ratio of variable to maximal fluorescence (F_v/F_m) during dehydration as a function of relative water content (RWC) in detached sun (open symbols) and shade (closed symbols) leaves of the three studied species. Each point represents the mean value with standard deviations on the same 5 different leaves maintained at room conditions (20–22 °C, and 70–80% RH) and very low light (5–10 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$).

(87% RWC) and were rehydrated overnight, their photochemical efficiency was recovered (Points), even on attaining 47% RWC (rhombus) with about 0.5 F_v/F_m they almost recovered. This did not happen when they were rehydrated after attaining lower values of RWC, 38%, (squares). In shade leaves the rehydration did not produce any recuperation, since when the leaves showed values which deviated from the normal ones (F_v/F_m about 0.6) the RWC was as low as 29%, and, when they were rehydrated, F_v/F_m values remained lower than the previous ones (crosses).

The analysis of the electrolyte leakage in sun leaves showed that the membranes started to be damaged at about 40% of RWC (Fig. 6).

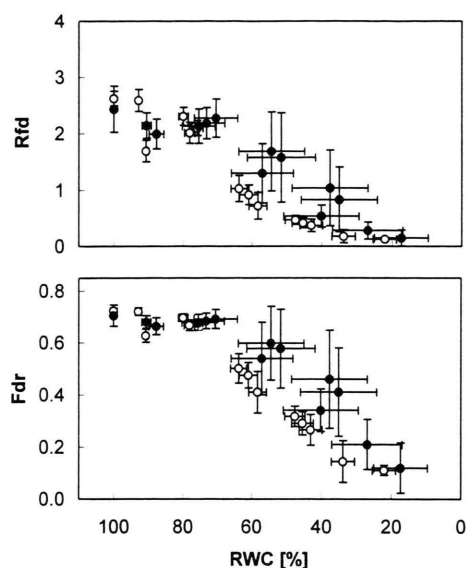


Fig. 3. Values of slow kinetic parameters as a function of relative water content (RWC) in detached sun (*open symbols*) and shade (*closed symbols*) leaves of *Persea indica*. Each point represents the mean value with standard deviations on the same 5 different leaves maintained at room conditions (20–22 °C, and 70–80% RH) and very low light ($5\text{--}10\ \mu\text{mol photon m}^{-2}\ \text{s}^{-1}$). $Rfd = F_d/F_s = F_m - F_s/F_s$ and $Fdr = F_d/F_m = F_m - F_s/F_m$.

Discussion

The effect of dehydration on the photochemical efficiency of PSII was different according to the species and type of leaves: Shade leaves of the three studied species were better adapted to this kind of treatment (they were not affected until 40% RWC) than corresponding sun leaves and among the latter that of *M. faya* were the most resistant (until 52% RWC while *P. indica* and *L. azorica* only until 90% RWC). In other species, for instance *Quercus petraea*, in a similar experiment the F_v/F_m ratio did not decrease until near 20% RWC (Epron and Dreyer, 1992). This indicates the great susceptibility of laurel forest trees to water deficit in their leaves. It could be thought that the differences between sun and shade leaves were due to different rates of dehydration in both leaf types, but as was shown in Fig. 1 the two rates were very similar so that no more possibility of damage exists due to longer dehydration time in either of them.

On the contrary the slow kinetic parameters showed similar values in sun and shade leaves of

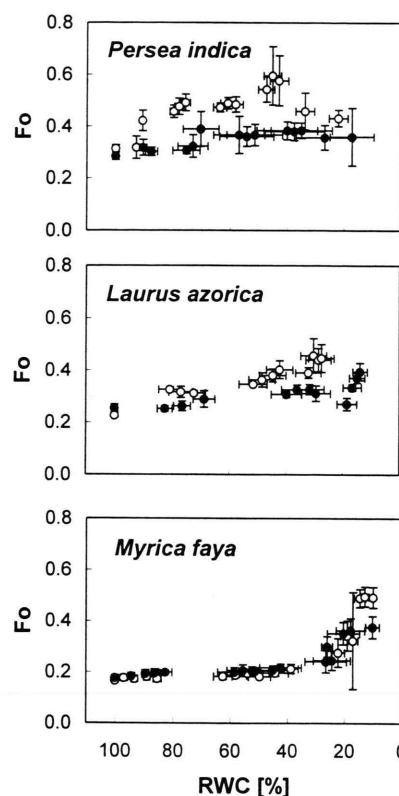


Fig. 4. Values of the minimal fluorescence (F_o) as a function of relative water content (RWC) in detached sun (*open symbols*) and shade (*closed symbols*) leaves of the three studied species. Each point represents the mean value with standard deviations on the same 5 different leaves maintained at room conditions (20–22 °C, and 70–80% RH) and very low light ($5\text{--}10\ \mu\text{mol photon m}^{-2}\ \text{s}^{-1}$).

the three species decreasing at relatively high values of RWC (about 70%). This value is in accordance with the findings of Kaiser (1987) and Cornic *et al.* (1989) in which the dehydration was obtained either rapidly by cutting the leaves (as in our case) or slowly by withholding water supply. These and other experiments all reviewed by Cornic *et al.* (1992) indicated that 30% leaf water deficit appears to be the limit under which the photosynthetic biochemistry is significantly affected not showing differences among different types of plants.

The membranes did not suffer any damage (determined by the analysis of the electrolyte leakage) until the leaves attained 40% of RWC so that after the PSII and also the biochemical reactions

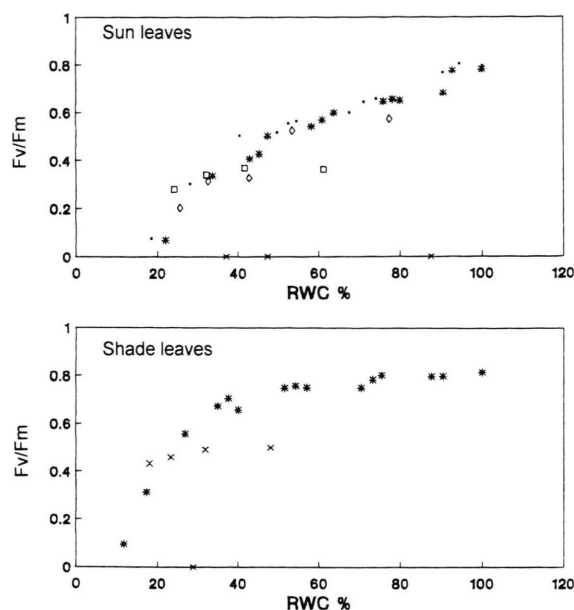


Fig. 5. Effect of dehydration and rehydration in F_v/F_m ratio on detached sun and shade leaves of *Persea indica*. Asterisks, effect of the RWC decrease as shown in Fig. 2. Points, rhombus, squares and crosses, effect of the rehydration after having been desiccated at the RWC levels marked in the abscissa (87, 47, 37 and 29% for the points, rhombus, squares and crosses respectively). Each point represents the mean value on the same 5 different leaves maintained at room conditions (20–22 °C, and 70–80% RH) and very low light (5–10 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$). Rehydration was obtained by keeping the leaves to water saturation during the night.

were affected. This parameter has been used as a measure of drought tolerance (Pramachandra *et al.*, 1990; Vasquez-Tello *et al.*, 1990) and Epron and Dreyer (1992) found that in *Quercus petraea* the membrane started to suffer damage, determined by this technique, at the same value (40% RWC) as in our plants, although the decrease in the values of F_v/F_m was produced at a higher value, as we mentioned previously. So in our case the only studied parameter which is influenced by the species and leaf conditions is the F_v/F_m ratio.

The high light adaptation of leaves is usually associated with a higher stress tolerance (Lichtenhaler, 1996) so our results seem to be in contrast to that. But the light adaptation capacity of plants is genetically fixed and we should not forget that the studied trees are adapted to live in the very wet and shady conditions where the laurel forest is distributed (Hollermann, 1981). Nevertheless the

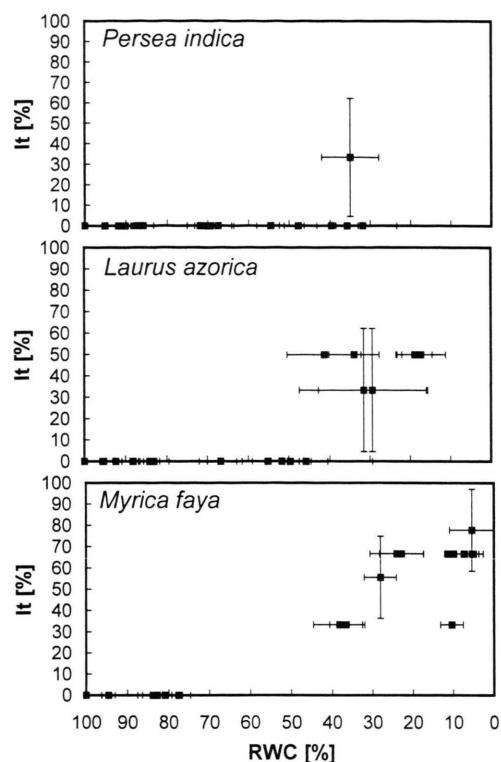


Fig. 6. Index of injury (I_t %) variation during dehydration as a function of relative water content (RWC) in detached sun leaves of the three studied species. Each point represents the mean value with standard deviations on 5 different leaves maintained at room conditions (20–22 °C, and 70–80% RH) and very low light (5–10 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$).

leaves which develop in the upper canopy have to cope with strong light conditions during clear day periods so only species as those studied, which can modify their morphology to adapt to the changing conditions along the canopy, are able to grow in this dense forest (Morales *et al.*, 1996a). The fact that the photosynthetic apparatus of sun leaves starts to be affected at much lower water deficits than shade leaves indicates that the mentioned changes experienced in the sun leaves, including the chlorophyll contents and antenna size, could bring about a greater susceptibility to damage in the antenna pigments, as shown by the increase of F_0 levels (It is known that the F_0 level reflects the emission of light-harvesting pigments when all reaction centers of PSII are in open state, Govindjee and Papageorgiou, 1971), nevertheless they would be better adapted to endure higher levels of light.

In fact, in our parallel studies in this forest we have found a decrease in the F_v/F_m ratio in sun leaves of these trees during days with very strong radiation levels, together with changes in the xanthophyll cycle pigment contents (Morales *et al.*, 1997), but the normal values of F_v/F_m were always recuperated during the night, therefore no permanent damage was produced.

In similar experiments with sun and shade leaves of the same species in relation to high and low temperature resistance (González-Rodríguez, 1998) shade leaves of *Persea indica* and *Laurus azorica* were again more resistant, while no clear

differences were found between *M. faya* leaves, being this species adapted to live in more open areas. These results show again, the great diversity of responses to water stress of the photosynthetic apparatus, depending on the habitats and specialization of plant life forms.

Acknowledgments

This work has been done thanks to the financial help of DGICYT (Spanish Government) project n° PB94-0580 and "Viceconsejería de Educación" (Canarian Government). Thanks to Neil Abrey for his help with the English style.

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